Pfeifer, David

From: Wang, Ning <nwang@usgs.gov>
Sent: Friday, June 14, 2013 12:14 PM
To: Vincent, Angela
Cc: Dennis McIntyre; Chris Skalski; Chris Tarr; Pfeifer, David; Bauer, Candice
Subject: Re: Franklin WER study plan

Angela,

Please see my following comments in red text.

All -

I received a phone call from Dennis today. Dennis confirmed my understanding of the procedure for measuring temperature, pH, DO and conductivity. DO, pH, and temperature are measured daily at every test concentration (i.e., in one of the four replicates per test concentration) and in one of the four replicates for the site water control (12% effluent, 88% upstream GMR water) and for the moderately hard water control.

Wang: How is that possible to measure the WQ DAILY for the mussel test? a typo? I suggest measuring temperature in the temperature-control water bath daily or in a beaker containing water without mussels daily, and measuring conductivity, pH, Do, hardness, alkalinity, and ammonia at the beginning of the test (using remaining water for each exposure concentrations after filling out all replicate beakers) and at the end of test (using composite sample from replicate beakers for a concentration after mussel survival determination). While it is good to measure the conductivity (or ammonia if high in test water) at every test concentration, it is OK to measure other WQ only at control, medium, and high concentrations.

Specific conductance is measured at the beginning of the test on the composite samples before distribution to the individual beakers and at the termination of the 96-hour acute testing. As Dennis mentioned, the probe for specific conductance is too large to use during the 96-hour tests, which is why this parameter is measured at the beginning and end of testing.

Wang: Is specific conductance the conductivity mentioned above? If yes, do no need this step if follow the above suggestion.

Ammonia will be measured at the start and at the end of 96-hour toxicity tests with juvenile mussel (L. fasciola) in the test water that contains field collected water. Hardness and alkalinity are determined in the high test concentration and in the primary and secondary control water at the beginning of the test.

Wang: Do no need this step if follow the above suggestion #1.

Dennis mentioned that the sodium chloride reference test will be run concurrently with the 96-hour acute toxicity testing. That is, a sub-sample of the batch of Lampsilis fasciola received will be used for the sodium chloride reference test. According to Dennis, the next batch of L. fasciola should be available by late June/early July.

Wang: It would be good to measure the chloride or sodium at all exposure concentrations at the beginning of the test. This test result would provide additional information for the EPA Region 5 for chloride criteria development.

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Ning Wang
Research Fish Biologist, Ph.D.
Columbia Environmental Research Center
U.S. Geological Survey
4200 New Haven Rd.
Columbia, MO 65201
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573/441-2946, 573/876-1896 (fax)
nwang@usgs.gov, http://www.cerc.usgs.gov/

On Fri, Jun 14, 2013 at 11:25 AM, Vincent, Angela <vincent.angela@epa.gov> wrote:

All -

I received a phone call from Dennis today. Dennis confirmed my understanding of the procedure for measuring temperature, pH, DO and conductivity. DO, pH, and temperature are measured daily at every test concentration (i.e., in one of the four replicates per test concentration) and in one of the four replicates for the site water control (12% effluent, 88% upstream GMR water) and for the moderately hard water control.

Specific conductance is measured at the beginning of the test on the composite samples before distribution to the individual beakers and at the termination of the 96-hour acute testing. As Dennis mentioned, the probe for specific conductance is too large to use during the 96-hour tests, which is why this parameter is measured at the beginning and end of testing.

Ammonia will be measured at the start and at the end of 96-hour toxicity tests with juvenile mussel (L. fasciola) in the test water that contains field collected water. Hardness and alkalinity are determined in the high test concentration and in the primary and secondary control water at the beginning of the test.

Dennis mentioned that the sodium chloride reference test will be run concurrently with the 96-hour acute toxicity testing. That is, a sub-sample of the batch of Lampsilis fasciola received will be used for the sodium chloride reference test. According to Dennis, the next batch of L. fasciola should be available by late June/early July.

Dennis said he'll make final revisions to the Study Plan based on USEPA's and USGS's communications with him.

If anyone has additional comments, please let Dennis and/or I know. Dennis, I suggest including a note in the Study Plan that L. fasciola will be used instead of V. fabalis. Please also feel free to edit/respond to the above summary, if necessary. Thanks.

Angela

Angela Vincent vincent.angela@epa.gov
U.S. EPA, Region 5 312-353-9715
Water Quality Branch (WQ-16J) 312-697-2633 (fax)
77 W. Jackson Blvd
Chicago, IL 60604

-----Original Message-----
From: Vincent, Angela
Sent: Wednesday, May 29, 2013 1:13 PM
To: 'Dennis McIntyre'
Cc: Chris Skalski; Chris Tarr; Wang, Ning
Subject: RE: Franklin WER study plan

Dennis - Thanks for making these revisions. A few additional questions about the experimental design.

Page 13 of the study plan states that "Temperature, pH, dissolved oxygen and conductivity will be measured at

selected times in the chemistry control replicate for each test concentration in each toxicity test."

I understand that each of these parameters (temperature, pH, DO, and conductivity) is measured via probe. Does this mean that one of the four replicates per test concentration (including controls) contains the appropriate probes for measurement of these parameters (i.e., temperature, pH, and DO)? I understand that there are four test chambers per test concentration with 5 mussels per test concentration (i.e., 4 replicates per test concentration). It appears specific conductance is measured at the beginning of testing from a composite sample before distribution to individual test beakers (11.2.12). Dennis, you also mentioned that specific conductance would be measured at the end of toxicity testing.

11.2.12 (XI. Procedure for mussel toxicity tests, Appendix D) states: "Hardness and alkalinity are determined in the high test concentration and control water at the beginning of the test. Specific conductance is measured at the beginning of the test on the composite samples before distribution to the individual test beakers. Dissolved oxygen, pH, and temperature measurements are made daily in every test concentration and control."

Please clarify whether 11.2.12 is the procedure for water quality measurements during mussel toxicity tests and whether the above paragraph (my understanding) is also accurate.

The above description at 11.2.12 is consistent with what Dennis and I discussed on the phone. The ASTM 2006 method for conducting laboratory toxicity tests with freshwater mussels says that ammonia should also be checked in toxicity tests. Ning - Do you agree that ammonia should be measured at the start of toxicity tests with juvenile mussel? If yes, the description at 11.2.12 should be modified, as appropriate.

Appendix D, XI. Procedure for juvenile mussel toxicity testing says at 11.2.3. "Juvenile mussels, observed as active, are placed in test chambers and subject to test conditions for 96+/-1 hours." How are juvenile mussels observed as active before placement into test chambers at the start of acute toxicity testing? Dennis, is this the sodium chloride reference test that you mentioned?

Page 12 of the Study Plan: Are spiked lab water test solutions and spiked simulated downstream water test solutions both equilibrated for the same amount of time (2 hrs? 3 hrs?)?

Minor additional revisions -
1. ASTM 2007a is the same citation as ASTM 2006. Please update this citation in Appendix C. (ASTM 2006 was re-approved in 2013.) 2. Chart on page 9 refers to DMW. This should be MHW.

Ning - If you have any other comments, please let Dennis or I know. Thanks.

Angela Vincent
312-353-9715

Angela Vincent vincent.angela@epa.gov
U.S. EPA, Region 5 312-353-9715
Water Quality Branch (WQ-16J) 312-697-2633 (fax)
77 W. Jackson Blvd
Chicago, IL 60604

-----Original Message-----
From: Dennis McIntyre [mailto:dmcintyre@glec.com]
Sent: Thursday, May 23, 2013 2:56 PM

To: Vincent, Angela
Cc: Chris Skalski; Chris Tarr; Wang, Ning
Subject: Re: Franklin WER study plan

Dear Angela and others,
Angela and I discussed her and Ning's comments that are listed in her message below. I have made the following changes to the attached plan:

Study site map was added to Appendix A which is referred to on the top of page 3.

DMW typo changed to MHW (top of page 9)

Added dissecting microscope detail to endpoint measurement in table on page 6.

Added one more test concentration (12.3 µg/L) at the low end of the test range (also in table on page 6).

The addition of one more test concentration at the low end is an attempt to bracket the LC50. If we have plenty of juvenile mussels, we may add another one (8.6 µg/L). We are heavy at the high end in the event the WER has the possibility to increase the Cu criterion toward that end.

I just talked to Jess Jones at FWS and he said they have much more wavy-rayed lampmussels right now (Lampsilis fasciola) than Villosa iris and were hoping that we could use that species. Ning, I looked at your 2007 paper and L. fasciola (Cu LC50 22 - 25 µg/L) is pretty close to the V. iris (Cu LC50 17 µg/L). I told him I would need to get approval to make the switch in species. What do you guys think?
Dennis

On 5/22/2013 9:52 PM, Vincent, Angela wrote:
> Hi Dennis -
>
> I have questions concerning the proposed acute toxicity testing with juvenile V. iris (rainbow mussel). I would like to clarify the experimental design/setup for acute mussel toxicity testing as described in the study plan. According to ASTM 2006 (Conducting laboratory toxicity tests with freshwater mussels), toxicity tests should include a negative control and appropriate solvent or dilution water controls. Here's what I understand of the experimental design based on information provided in the study plan and ASTM 2006:
>
> The lab reference water dilution control is lab reference water (88% moderately hard lab water/ 12% Scioto River water) mixed with Millipore water at a specific ratio. Let's say, hypothetically, that the ratio is 9 parts LRW: 1 part Millipore water. This dilution water is then used to prepare a serial dilution (6 or 7 test concentrations) of the lab reference water spiked with cupric sulfate five hydrate. For example, if test concentration #1 is 9 parts LRW: 1 part stock solution, where the stock solution is Millipore water spiked with cupric sulfate five hydrate, the next concentration, concentration #2 could be 50% of test concentration #1 and 50% of 9 parts LRW: 1 part Millipore water. Therefore, in this setup you have one dilution control (i.e., 9 parts LRW: 1 part Millipore water) with three replicates of the dilution control water in the experiment. Each test chamber has five test specimens with 4 test chambers per treatment (i.e., 20 test specimens per test concentration or control dilution water).
>
> A dilution water control for the simulated downstream water (e.g., 88% upstream Great Miami River water/12% effluent) would be prepared in the same manner as described above for lab reference water. The proposed test concentrations (page 6 of the Study Plan) are 17.6, 25.2, 36, 51.4, 73.5, 105 and 150 ug/L copper sulfate. Therefore, a serial dilution with simulated downstream water at the indicated concentrations of copper sulfate is required. I spoke with Ning Wang and we both have the same question. Do these test concentrations

bracket (or include) the estimated EC50? Is a 48-hour screening test necessary to determine appropriate test concentrations? Please explain how the aforementioned test concentrations were determined. Hardness and dissolved organic carbon influence copper toxicity and should be considered in determining appropriate test concentrations.
>
> Please let me know if my explanation of the experimental setup is clear/accurate or requires revision.
>
> We also need clarification as to which water quality parameters are measured, and when and how they are measured. For example, according to ASTM, dissolved oxygen, pH, ammonia, hardness, alkalinity, and conductivity should be measured at the start and end of acute toxicity tests. It seems logical that DOC should also be measured at least at the start and end of 96-hour acute copper toxicity tests. According to ASTM 2006, calcium, magnesium, sodium, potassium, chloride, and sulfate should be measured in the dilution water. As for sample collection, a composite sample from individual replicates at a specific test concentration may be desirable. According to ASTM, in static and renewal tests, temperature should be measured at least hourly or the maximum and minimum temperatures must be measured daily. Dissolved oxygen (and pH and conductivity) can be measured with a probe during acute toxicity testing and should be maintained above 4 mg/L throughout the test.
>
> Other additions/revisions -
>
> 1. Please incorporate the attached site map into the study plan. This map is a helpful visual of the study area.
> 2. I confirmed with Ning that ASTM 2007a is the same citation as ASTM 2006. Please update this internal citation and the works cited. ASTM 2006 was re-approved in 2013.
> 3. On page 9 of the study plan, is MHW (moderately hard water) the same as DMW? Please explain.
> 4. A dissecting microscope should be listed under equipment and supplies of the SOP for acute toxicity tests with newly transformed juvenile rainbow mussels. This microscope is needed to determine if juvenile mussels exhibit foot movement for classification as alive or dead.
>
> American Society for Testing and Materials. 2006 (Re-approved 2013). Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. In Annual Book of ASTM Standards, Vol 11.06 Philadephia, PA pp 1393-1444.
>
> Please give me a call at your convenience (312-353-9715) to discuss any of the above comments. I am in the office tomorrow, Thursday. I am on furlough on Friday. Thanks.
>
>
> Angela Vincent vincent.angela@epa.gov
> U.S. EPA, Region 5 312-353-9715
> Water Quality Branch (WQ-16J) 312-697-2633 (fax)
> 77 W. Jackson Blvd
> Chicago, IL 60604
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> -----Original Message-----
> From: Dennis McIntyre [mailto:dmcintyre@glec.com]
> Sent: Monday, May 20, 2013 1:39 PM
> To: Vincent, Angela
> Cc: Chris Skalski; Chris Tarr
> Subject: Re: Franklin WER study plan
>
> Angela,

> That is great - thanks.
> Dennis
> On 5/20/2013 2:31 PM, Vincent, Angela wrote:
>> Dennis - Thank you for providing the revised Franklin copper SSC study plan. I am working through my review at this time. I hope to get comments back to you by Tuesday or Wednesday of this week. Does that work for you? Thank you.
>>
>> Angela Vincent vincent.angela@epa.gov
>> U.S. EPA, Region 5 312-353-9715
>> Water Quality Branch (WQ-16J) 312-697-2633 (fax)
>> 77 W. Jackson Blvd
>> Chicago, IL 60604
>>
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>> -----Original Message-----
>> From: Dennis McIntyre [mailto:dmcintyre@glec.com]
>> Sent: Monday, May 13, 2013 10:28 AM
>> To: Chris Skalski; Vincent, Angela
>> Cc: Chris Tarr
>> Subject: Franklin WER study plan
>>
>> Angela and Chris,
>> It has been a few weeks since we last communicated about Franklin's WER study plan. We were to make changes to the plan to include the comments made by on the draft plan which included a test with a freshwater mussel. These changes are in the attached revised plan.
>>
>> I highlighted (in yellow) the relevant changes in the attached plan to make it easier to see what has been added/changed. Regarding the mussel test, we have been in communication (conference call and emails) with Chris Ingersoll and Ning Wang from USGS to make sure we have the current testing practices. Both Chris and Ning were very receptive to being involved and were very helpful in our discussions. Ning reviewed the parts of the attached plan that pertain to mussels.
>>
>> We have also been in contact with a source of mussels to use in the test. Jess Jones from the Freshwater Mollusk Conservation Center has agreed to supply us with juvenile rainbow mussels. This is the species USGS recommended. There is, however, a closing window of opportunity to obtain juveniles of this species this year and I am asking you to look over the plan fairly soon so we can get this testing in this spring. I do apologize for the rush.
>>
>> I will be a travel tomorrow through Thursday of this week, but I will be checking emails in the evening.
>> Kind Regards,
>> Dennis
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>> Dennis McIntyre
>> GLEC
>> 1295 King Avenue
>> Columbus, Ohio 43212
>> dmcintyre@glec.com
>> 614-487-1040
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Dennis McIntyre
GLEC
1295 King Avenue
Columbus, Ohio 43212
dmcintyre@glec.com
614-487-1040